2014

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Environmental Management (2014) 53:416–428
DOI 10.1007/s00267-013-0194-6

The Influence of Study Species Selection on Estimates of Pesticide Exposure in Free-Ranging Birds

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Received: 22 December 2012 / Accepted: 17 October 2013 / Published online: 31 October 2013

Abstract Field studies of pesticide effects on birds often utilize indicator species with the purpose of extrapolating to other avian taxa. Little guidance exists for choosing indicator species to monitor the presence and/or effects of contaminants that are labile in the environment or body, but are acutely toxic, such as anticholinesterase (anti-ChE) insecticides. Use of an indicator species that does not represent maximum exposure and/or effects could lead to inaccurate risk estimates. Our objective was to test the relevance of a priori selection of indicator species for a study on pesticide exposure to birds inhabiting fruit orchards. We used total plasma ChE activity and ChE reactivation to describe the variability in anti-ChE pesticide exposure among avian species in two conventionally managed fruit orchards. Of seven species included in statistical analyses, the less common species, chipping sparrow (*Spizella passerina*), showed the greatest percentage of exposed individuals and the greatest ChE depression, whereas the two most common species, American robins (*Turdus migratorius*) and gray catbirds (*Dumatella carolinensis*), did not show significant exposure. Due to their lower abundance, chipping sparrows would have been an unlikely choice for study. Our results show that selection of indicator species using traditionally accepted criteria such as abundance and ease of collection may not identify species that are at greatest risk. Our efforts also demonstrate the usefulness of conducting multiple-species pilot studies prior to initiating detailed studies on pesticide effects. A study such as ours can help focus research and resources on study species that are most appropriate.

Keywords Anticholinesterase pesticide · Birds · Exposure · Indicator species · Orchard

Introduction

Pesticides have been shown to have adverse effects on birds in the field, even after lawful applications (Mineau et al. 1999; Stinson et al. 1994; Stone 1979). Field studies can provide valuable information for assessing the hazards of pesticides to birds under conditions of operational use; however, because avian field studies can be resource intensive, their focus is often limited to one or a few targeted study species. These study species are utilized as biological indicators, and data collected on them are extrapolated to other avian species. Indicator species have been used in various conservation scenarios, and the indicator species concept may be used in wildlife toxicological field studies with the assumption that these species are similar or greater in their exposure and/or sensitivity than are other species (Greig-Smith 1990; Hardy 1990). However, not all species are necessarily equally capable of representing effects to all species. For example, studies

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have been conducted wherein adverse effects were not observed in the intended study species, but were noted in other species within the study sites (Millikin and Smith 1990; Powell, unpublished data), or adverse effects were not observed in a study species because of specific behaviors that reduced exposure (Johnson et al. 1976). Within agroecosystems, birds occupy numerous niches resulting in varying degrees of exposure to pesticides, and their sensitivities are also known to vary (Hill 1992; Smith 1987; Thompson et al. 1995; Wiemeyer and Sparling 1991). As a result, each species could differ in terms of its biological relevance as a sentinel of effects to other birds.

Methods of choosing indicator species have received attention in several areas of wildlife conservation, particularly in biological monitoring for determining the success of wildlife management and the maintenance of biodiversity (e.g., Andelman and Fagan 2000; Bani et al. 2006; Hutto 1998; Niemi and McDonald 2004; Thompson 2006). For contaminants, the use of indicators has focused on persistent chemicals and choice of indicator species that are used as gauges of environmental quality (i.e., biomonitors) or as markers of effects that are typically long-term on wildlife populations. Species used for these purposes are selected to represent changes in contaminant levels in the environment and so those species must be able to persist despite exposure (Landres et al. 1988; Moore 1966). However, little empirical data or guidance exists for choosing indicator species for contaminant studies with labile chemicals that have the potential for short-term but highly acute effects (Greig-Smith 1990). Indicator species used in field studies with pesticides are often selected on the basis of abundance and convenience in collecting data with that species (e.g., Millikin and Smith 1990; see also Bouvier et al. 2005; Burgess et al. 1999; Greig-Smith 1990; Jones 2003; Patnode and White 1991; Rondeau and DesGranges 1995). Selection of a species based on its abundance may provide greater numbers of individuals for study and analysis, but a species may be abundant because it is less sensitive or does not receive the exposure necessary to result in a toxic effect. Species that utilize nest boxes, which are frequently used in bird contaminant studies, may be more easily captured, monitored, or manipulated (Jones 2003), but these species have potentially lower exposure resulting from occupying a more protected nest site compared to species with more open nest sites.

Given, the paucity of information on relative sensitivities among species and the complexity of exposure that can occur in the field, the selection of an appropriate study species is imperative for understanding the true extent of the effects of pesticides to birds in the field. Our objective was to test the relevance of a priori selection of indicator species for a study on pesticide exposure to birds inhabiting fruit orchards.

Methods

All animal-handling procedures used in our study were approved by the Animal Care and Use Committees of U.S. Geological Survey Patuxent Wildlife Research Center, Laurel, MD and the University of Maryland, College Park, MD.

Study Sites

Field sampling was conducted in 1999 and 2000 at two pesticide-treated orchards near Kearneysville, West Virginia: the U.S. Department of Agriculture Appalachian Fruit Research Station and the West Virginia University Kearneysville Tree Fruit Research and Education Center (hereafter referred to as the USDA-treated site and the WVU-treated site, respectively). The USDA- and WVU-treated sites were approximately 202 and 63 ha in size, respectively, and contained several varieties of apple (*Malus domestica*) and peach (*Prunus persica*) trees. The USDA-treated site also contained small sections of pear (*Pyrus communis*) trees. Both treated sites included non-orchard habitat within and around them (other types of agricultural fields, pastures, wooded areas, residential areas, and roads); a small commercial orchard also bordered the USDA-treated site. During our sampling periods, non-orchard agricultural fields within the treated sites received no pesticide applications. Both treated sites were used for agricultural research, but most trees received conventional pest management at the time of our study. Both treated sites generally were divided into sections by crop/variety and agricultural experimental treatment and each section received pesticides approximately every 2 weeks on its own schedule. The two treated sites differed in that all sections at the WVU-treated site were generally treated at once whereas sections at the USDA-treated site were treated on different days such that new applications of pesticides occurred within that site every few days.

Organophosphates (OPs) and carbamates (CBs) were applied in both treated sites in both years. Principal OPs applied in both years were azinphos methyl (*O*,*O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3-(4*H*)-yl)methyl] phosphorodithioate applied at 0.31–0.75 lbs ai/acre [0.35–0.84 kg/ha]); phosmet (*S*-[(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl] *O*,*O*-dimethyl phosphorothioate; applied at 0.53–3.50 lbs ai/acre [0.59–3.92 kg/ha]); and additional use of methyl parathion (*O*,*O* dimethyl *O*-(4-nitrophenyl) phosphorodithioate; applied at 0.31–0.75 lbs ai/acre acre [0.35–0.84 kg/ha]) in 1999. In 2000, chlorpyrifos (*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) was used at both sites and applied at 0.62–3.00 lbs ai/acre (0.70–3.63 kg/ha). Malathion (diethyl (dimethoxythiophosphorylthio) succinate, applied at 0.94 lbs ai/acre [1.05 kg/

ha]) was used both years at the USDA site only. The principal CB insecticides used in both years were methomyl (*S*-methyl *N*-[(methylcarbamoyl)oxy] thioacetimidate applied at 0.34–0.68 lbs ai/acre [0.38–0.76 kg/ha]) and carbaryl (1-naphthyl methylcarbamate applied at 0.52–2.50 lbs ai/acre [0.58–2.81 kg/ha]). There was minor use of oxamyl (*S*-methyl *N*′,*N*′-dimethyl-*N*-(methylcarbamoyloxy)-1-thio-oxamimidate applied at 0.75 lbs ai acre [0.84 kg/ha]) at USDA in 2000. These compounds were applied in the following formulations: Guthion 50WP (azinphos methyl, Bayer Corp.), Malathion 57EC (malathion, Platte Chemical), Penncap-M (methyl parathion, ELF Atochem, Inc.), Imidan 70W (phosmet, Gowan Company), Lannate LV and Lannate SP (methomyl, DuPont Corp.), Sevin XLR (carbaryl, Rhone-Poulenc), Lorsban 50W (chlorpyrifos, Gowan Company) and Lorsban 4E (chlorpyrifos, Dow Agrosciences), and Vydate-L (oxamyl, DuPont). Sampling dates and anti-cholinesterase (anti-ChE) insecticides applied at the treated sites within 14 days before the first day of each sampling period are listed in Table 1. In 1999, 17 fungicides, eight herbicides, and 13 other non-anti-ChE insecticides or acaricides were also applied throughout the field season. In 2000, 14 fungicides, six herbicides, and 12 other non-anti-ChE insecticides or acaricides were also applied. In addition to these chemicals, adjuvants, fertilizers, and plant growth regulators were applied (see Borges 2002 for list).

Sampling also was conducted at two reference sites during 2000 and 2001. No suitable unsprayed orchard could be found within a reasonable distance of the USDA- or WVU-treated sites, so the principal reference site was located at the U.S. Fish and Wildlife Service, Patuxent Research Refuge (PRR) in Laurel, Maryland. PRR is approximately 5,260 ha, and consists of various avian habitats. Specifically, sampling was conducted in shrub, fields bordered by wooded areas, wooded areas, grass areas near buildings, and along wetland habitats. PRR received limited applications of herbicides during years that sampling took place but not within the sampling areas. Some species were present in insufficient numbers at PRR, so a

Table 1 Anti-ChEs present at USDA and WVU during sampling dates in 1999 and 2000

1999		2000	
Sampling dates	Anti-ChEs appl. within previous 14 days[a]	Sampling dates	Anti-ChEs appl. within previous 14 days[a]
USDA		USDA	
May 21–22	AM, PH, CB, MP	June 5–7	PH, AM, CB, CL, MA, MM
May 25	AM, PH, MP	June 12	AM, CB, PH, MA, MM, (MM + PH)
June 3, 5–6	(AM + MM), AM, MA, PH	June 19–20	CL, PH, AM, CB, MM, MA
June 11–13	AM, MA, MM	June 26–27	(CL + OX), MM, AM, CL, MA, PH
June 16	PH, AM, MA, MM	July 2–4	PH, CB, CL, MA, MM, (MM + AM + OX)
June 18–20	MP, AM, MA, MM, PH	July 9–11	PH, CB, MA, MM
June 21–22	PH, AM, MA, MM	July 17–18	PH, CB, MM
July 3, 5–6	MM	WVU	
July 13	PH	May 29–31	AM, PH, (PH + AM + MM)
July 17–18	CB, PH, MM	June 1–3	CL, PH, (AM + MM), (PH + AM + MM), AM
July 29	PH, CB, MP, MA	June 8–9	CL, PH, (AM + MM), (PH + AM + MM), AM
August 13–14	CB, MM, MP	June 13–14	CL, PH, (AM + MM), (PH + AM + MM), AM
WVU		June 16–18	PH, (AM + MM), (PH + CL), (PH + AM + MM)
May 26–28	AM, MM, PH	June 22–23	PH, (AM + MM), (PH + CL), (PH + AM + MM)
June 11–13	(MP + MM), MP, PH	June 27–28	PH, (AM + MM), (PH + CL), (PH + AM + MM)
June 25–27	CB, MP, PH	June 29–July 1	CL, PH, (PH + CL), (PH + AM + MM)
July 8–11	AM, CB, PH	July 6–7	CL, PH, (PH + CL), (PH + AM + MM)
July 30–August 1	AM, CB, PH	July 11–12	CL, PH, (PH + CL), (PH + AM + MM)
August 7–8	AM, CB, PH	July 13–15	AM, CB, PH, (PH + CL), (PH + AM + MM)
August 12–13	CB, (MP + MM), PH	July 20–21	AM, CB, PH, (PH + CL), (PH + AM + MM)
		July 25–26	AM, CB, PH, (PH + CL), (PH + AM + MM)
		July 27–29	CB, CL, PH, (CL + PH), (PH + AM + MM)
		August 3–4	CB, CL, PH, (CL + PH), (PH + AM + MM)

AM azinphos methyl, *PH* phosmet, *CB* carbaryl, *MP* methyl parathion, *MA* malathion, *MM* methomyl, *CL* chlorpyrifos, *OX* oxamyl

[a] Anti-ChEs enclosed in parentheses were applied together as a mixture

second reference site was located in Paw Paw, West Virginia. This site consisted of approximately 200 ha of privately owned land containing large hay or fallow fields interspersed with patches of abandoned apple and peach orchards (<1–3 ha in size each). Local residents around the site stated that pesticides were not applied to or around the sampling areas. Because the site consisted extensively of hayfields, the species composition differed from our treated sites. Therefore, this location was not used as the primary reference site.

Sampling Methods

When entering the orchards during the Restricted Entry Intervals, all personnel wore the required personal protective equipment (USEPA 1992 [40 CFR §170.240]). In 1999, birds were captured at the USDA- and WVU-treated sites from mid-May to mid-August. At both sites in 1999, mist nets were placed in three treated sections of the site, and operated for two to four consecutive days beginning no sooner than 4 h after pesticide applications (due to restrictions on re-entry and optimizing timing of bird capture during the day). In 2000, the sampling scheme differed between the two sites because pesticides were applied to the entirety of the WVU-treated site once every 2 weeks, whereas the USDA-treated site received pesticide treatments in a more piecemeal fashion. At USDA, pesticides were applied to different areas of the orchard every few days, and each treated area of the orchard received pesticides about once every 2 weeks. Sampling was conducted from mid-May to early August at the WVU-treated site. Nets were placed in four random locations around the WVU-treated site, and capture efforts were made at three times relative to a pesticide treatment: within 1 day prior, within 1 day after, and 1 week after. Sampling continued according to this schedule for five pesticide applications totaling 10 weeks. At the USDA-treated site, sampling was conducted between late-May and mid-July of that year. Mist nets were placed in four fixed areas of the site, and capture efforts took place for 2 days on a weekly basis. Sampling at USDA ceased due to low capture numbers after mid-July.

No suitable reference site could be found in 1999, so reference samples were not collected in that year. Birds were captured weekly at the PRR reference site during late-June through mid-August in 2000 and daily from late-May through early-August in 2001. Birds were captured between 19 and 25 July at the Paw Paw reference site in 2001. Nets were placed in areas where species of interest were seen or heard and were operated daily until capture success in those areas decreased.

In all years, mist nets were operated between 0600 and 0900 and between 1800 and 2000 h. Within the orchard sections, the nets were sited along a row of trees, and set up in a box-like arrangement on each side to catch birds flying in from all directions. At the PRR and Paw Paw reference sites, nets were similarly arranged as the habitat permitted. A wildlife caller (Johnny Stewart model 612-LR, Hunter's Specialties, Cedar Rapids, Iowa) playing Eastern screech owl (*Megascops asio*) calls was placed in the center of the nets to attract birds. Northern mockingbirds (*Mimus polyglottos*) and brown thrashers (*Toxostoma rufum*) appeared to be less reactive to the owl calls, so a recording of those species' songs was also played (songs were obtained from the Cornell Lab of Ornithology Library of Natural Sounds [now the Cornell Lab of Ornithology Macaulay Library]). Nets were also left open with no caller to capture birds that may have been deterred by the calls. Nets were checked for birds every 30 min. Birds were not marked at the treated sites, but if they showed evidence (e.g., matted feathers resulting from alcohol application) of having been sampled recently they were released. Birds were marked at the PRR and Paw Paw reference sites in 2001, and recaptures at that site were rare (26 out of 605 birds were recaptured), so it is probable that most of the samples taken at the treated sites and from the PRR reference site in 2000 were from different individuals.

Blood samples were collected from all birds captured at the WVU- and USDA-treated sites, regardless of species, age, or sex; corresponding species and age groups were selectively captured at the PRR and Paw Paw reference sites. Blood was collected via brachial venipuncture into heparinized capillary tubes. Samples were placed on ice and centrifuged on site within 1 h of collection at $13{,}500 \times g$ for 10 min. The plasma was transferred to an unheparinized collection tube and immediately frozen in liquid nitrogen. Plasma samples were stored in liquid nitrogen in the field (for less than 1 week) until they could be placed in permanent storage at -80 °C.

Exposure Determination

Two types of ChE analyses were used to determine exposure of birds to anti-ChEs: total ChE measurement, which requires comparison to threshold values calculated from birds captured at reference sites, and ChE reactivation, which compares ChE activity in the same sample with and without a reactivation treatment to restore ChE activity to a non-inhibited state. Ideally, all samples would have been subjected to both the total ChE activity assay as well as the ChE reactivation assays. However, since plasma ChE activity is known to be highly variable, some exposures are more difficult to detect with comparison of total ChE activity to threshold values, and in some cases a sufficient number of reference samples could not be collected for some species. Reactivation techniques can capture more

subtle changes and do not require comparison to threshold values calculated from reference birds, but analyses for both OP and CB reactivation require more plasma than we were able to collect from some birds. Therefore, a decision was made about each plasma sample, based on its volume, to perform either the total ChE activity or ChE reactivation analysis, or both when possible. Comparison of these techniques in their ability to detect ChE inhibition and the effect of both types of ChE analyses on the results of this study are discussed below.

ChE analyses were performed using a Spectramax 250 96-well microplate reader (Molecular Devices, Sunnyvale, CA). All reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Analyses were conducted using the methods of Ellman et al. (1961) as modified for a 96-well microplate reader (Gard and Hooper 1993). Total ChE activity, expressed as micromoles of substrate (acetylthiocholine iodide) hydrolyzed per minute per milliliter of plasma (μmol/min/mL), was calculated as the mean of triplicate assays. ChE activity of control serum (Accutrol® control serum, Sigma-Aldrich Corp.) was analyzed at the beginning of each day and with every assay to assure the consistency of reagents and buffers.

Threshold values for determining exposure in total ChE assays were calculated as the value two standard deviations below the mean reference total ChE value for each age class (adult or juvenile) of each species (Hill and Fleming 1982). Threshold values for each age class of each species were determined from data pooled across both years and reference sites, excluding samples exhibiting ≥10 % reactivation (see below) and/or total ChE activity of <0.30 μmol/min/mL. Data were separated by age because total ChE activity is known to vary by age. Sufficient numbers of reference birds were not captured for brown thrashers, blue jays (*Cyanocitta cristata*), common grackles (*Quiscalus quiscula*), song sparrows (*Melospiza melodia*), and house sparrows (*Passer domesticus*). Threshold values for these species were calculated from reference data provided by Dr. Michael Hooper (USGS, unpublished data), who used the same ChE assay procedures. Means and standard deviations were compared for similar species captured in this study and Dr. Hooper's studies, and were found to be similar (e.g., means differed by 3–9 %, standard deviations differed by 4–5 %).

Both OP- and CB-specific reactivation analyses were performed on plasma samples that were of sufficient volume. If only one type of reactivation analysis could be performed, the type was chosen based on the class of chemical most recently applied prior to each bird's capture. The oxime 2-PAM (pyridine-2-aldoxime methochloride) was used for reactivation of OP-inhibited ChE in our samples (see Hooper et al. 1989). CB-inhibited samples were reactivated using the methods of Hunt and Hooper (1993). Total ChE was considered to be reactivatable if mean activity of the 2-PAM-treated or CB-extracted aliquot was ≥10 % higher than the mean activity of the non-treated aliquot or pre-extraction sample and if a one-tailed Student's t test indicated that the difference between their means was significant at $\alpha = 0.05$.

A bird was considered to have had anti-ChE exposure if its total ChE activity was below its critical threshold value and/or if its total ChE was reactivatable by ≥10 %. A paired Z test for proportions (of exposed to unexposed birds) was carried out to determine the level of agreement between the results of the two tests for exposure. Comparability was tested using the criterion that at least 90 % of the pairwise tests had to agree (i.e., H_0: proportion of tests in agreement ≥0.9). Agreement in the results of the two tests was achieved ($Z = 0.80$, $P = 0.57$, $n = 299$).

Statistical Analyses

All statistical analyses were performed using SAS software (SAS version 8.2, SAS Institute, Cary, NC). Two types of data analysis were performed to determine if any differences in exposure existed among species: (1) comparisons of the frequency of individuals categorized as exposed as described above, and (2) comparisons of mean total ChE activity for each species between the treated-orchard sites and the reference sites. All tests were restricted to those species for which ≥10 individuals were captured. Tests of exposure frequency were performed using χ^2 and Fisher's exact tests with experiment-wise $\alpha = 0.05$. A Bonferroni adjustment was applied when multiple tests were performed. Mean ChE activities were compared to test differences among species for which sample sizes were of adequate size. Analyses were performed using ANOVA, and assumptions were met by log-transforming the data or partitioning error variance among groups with similar variance where necessary. Comparisons between orchard and reference sites within each species were performed using Tukey–Kramer HSD tests or Bonferroni adjustment.

Results

Species Studied and Overall Exposure

In 1999 and 2000, 306 and 814 individual birds, respectively, comprising 26 species, were captured and used in our study (Tables 2, 3). Twenty-four additional species were either observed at the sites and not captured, or were captured but excluded because insufficient numbers of reference birds were captured. These included: Northern bobwhite (*Colinus virginianus*), sharp-shinned hawk (*Accipiter striatus*), American kestrel (*Falco sparverius*), mourning dove (*Zenaida macroura*), common nighthawk

Table 2 Percentage of individuals exposed by species, site, and year

Common names	Scientific names	1999			2000			Reference[a], % (*n*)
		WVU[a], % (*n*)	USDA[a], % (*n*)	Total[a], % (*n*)	WVU[a], % (*n*)	USDA[a], % (*n*)	Total[a], % (*n*)	
American robin	*Turdus migratorius*	31 (62)	27 (30)	29 (92)	16 (248)	12 (41)	16 (289)	4 (53)
Gray catbird	*Dumatella carolinensis*	21 (29)	16 (19)	19 (48)	8 (106)	5 (37)	8 (143)	5 (79)
House finch	*Haemorhous mexicanus*	20 (35)	13 (15)	18 (50)	7 (52)	27 (33)	15 (85)	2 (48)
American goldfinch	*Spinus tristis*	9 (11)	12 (17)	11 (28)	17 (29)	10 (20)	14 (49)	4 (45)
Northern cardinal	*Cardinalis cardinalis*	0 (8)	29 (7)	13 (15)	9 (23)	15 (20)	12 (43)	4 (71)
Chipping sparrow	*Spizella passerina*	36 (11)	75 (4)	47 (15)	17 (23)	25 (12)	20 (35)	2 (44)
Field sparrow	*Spizella pusilla*	8 (12)	20 (5)	12 (17)	21 (19)	0 (10)	14 (29)	3 (67)
Song sparrow	*Melospiza melodia*	0 (2)	50 (2)	25 (4)	29 (24)	20 (5)	28 (29)	0 (5)
Downy woodpecker	*Picoides pubescens*	25 (4)	0 (4)	13 (8)	0 (9)	0 (5)	0 (14)	5 (22)
Carolina chickadee	*Poecile carolinensis*	0 (2)	0 (8)	0 (10)	–	0 (8)	0 (8)	0 (37)
Common grackle	*Quiscalus quiscula*	0 (1)	0 (1)	0 (2)	19 (16)	–	19 (16)	4 (25)
Brown thrasher	*Toxostoma rufum*	33 (3)	0 (1)	25 (4)	33 (6)	20 (5)	27 (11)	17 (6)
Indigo bunting	*Passerina cyanea*	0 (3)	–	0 (3)	20 (5)	100 (1)	33 (6)	5 (42)
Red-eyed vireo	*Vireo olivaceus*	0 (1)	0 (2)	0 (3)	–	0 (3)	0 (3)	3 (59)
Wood thrush	*Hylocichla mustelina*	0 (1)	0 (2)	0 (3)	–	0 (3)	0 (3)	11 (19)
Blue jay	*Cyanocitta cristata*	50 (2)	0 (1)	33 (3)	50 (2)	–	50 (2)	0 (2)
Blue-gray gnatcatcher	*Polioptila caerulea*	100 (1)	–	100 (1)	–	100 (1)	100 (1)	0 (12)
House sparrow	*Passer domesticus*	–	–	–	19 (16)	–	19 (16)	–
Northern mockingbird	*Mimus polyglottos*	–	–	–	13 (8)	100 (1)	22 (9)	10 (10)
Tufted titmouse	*Baeolophus bicolor*	–	–	–	0 (2)	0 (6)	0 (8)	8 (36)
Carolina wren	*Thrytothorus ludovicianus*	–	–	–	0 (5)	0 (1)	0 (6)	0 (27)
Brown-headed cowbird	Molothrus ater	–	–	–	0 (3)	–	0 (3)	0 (2)
Worm-eating warbler	*Helmitheros vermivorum*	–	–	–	0 (2)	0 (1)	0 (3)	0 (9)
House wren	*Troglodytes aedon*	–	–	–	0 (2)	–	0 (2)	0 (12)
Scarlet tanager	*Piranga olivacea*	–	–	–	–	0 (1)	0 (1)	0 (17)
Yellow breasted chat	*Ictera virens*	–	–	–	0 (1)	–	0 (1)	0 (4)
Totals		22 (188)	19 (118)	21 (306)	14 (601)	13 (214)	14 (815)	4 (753)

[a] Percentages are rounded

(*Chordeiles minor*), ruby-throated hummingbird (*Archilochus colubris*), Northern flicker (*Colaptes auratus*), Eastern phoebe (*Sayornis phoebe*), great crested flycatcher (*Myiarchus crinitus*), Eastern kingbird (*Tyrranus tyrranus*), one unidentified flycatcher species, white-eyed vireo (*Vireo griseus*), American crow (*Corvus brachyrhynchos*), barn swallow (*Hirundo rustica*), Eastern bluebird (*Sialia sialis*), European starling (*Sturnus vulgaris*), cedar waxwing (*Bombycilla cedrorum*), yellow warbler (*Setophaga petechia*), common yellowthroat (*Geothylpis trichas*), Eastern towhee (*Pipilo erythrophthalmus*), grasshopper sparrow (*Ammodramus savannarum*), one unidentified sparrow species, rose-breasted grosbeak (*Pheucticus ludovicianus*), and orchard oriole (*Icterus spurious*).

Exposure was detected in all sampling events. The frequency of anti-ChE exposure among all birds included in the analyses did not differ between the two treated-orchard sites in either year ($\chi^2_1 = 0.24$, $P = 0.63$, $n = 308$ in 1999;

Table 3 Mean ChE activity ± SEM of each species captured at the orchard and reference sites in 1999 and 2000

Common names	1999			2000			Reference sites (*n*)
	WVU (*n*)	USDA (*n*)	Both sites (*n*)	WVU (*n*)	USDA (*n*)	Both sites (*n*)	
American robin	0.73 ± 0.04 (44)	0.82 ± 0.07 (25)	0.76 ± 0.03 (69)	0.96 ± 0.06 (84)	0.88 ± 0.05 (31)	0.93 ± 0.04 (115)	0.91 ± 0.05 (30)
American robin[a]	0.67 ± 0.04 (18)	0.80 ± 0.07 (5)	0.70 ± 0.03 (23)	0.74 ± 0.02 (163)	0.71 ± 0.08 (10)	0.74 ± 0.02 (173)	0.81 ± 0.05 (23)
House finch	1.11 ± 0.06 (35)	1.18 ± 0.07 (15)	1.12 ± 0.04 (50)	1.01 ± 0.04 (52)	1.00 ± 0.05 (33)	1.01 ± 0.03 (85)	1.03 ± 0.04 (48)
Gray catbird	0.93 ± 0.05 (29)	0.94 ± 0.06 (19)	0.93 ± 0.04 (48)	1.02 ± 0.04 (95)	1.07 ± 0.06 (36)	1.03 ± 0.03 (131)	1.03 ± 0.04 (60)
Gray catbird[a]	–	–	–	1.01 ± 0.06 (11)	0.63 (1)	0.98 ± 0.07 (12)	1.10 ± 0.06 (19)
American goldfinch	0.92 ± 0.07 (11)	0.84 ± 0.06 (17)	0.87 ± 0.05* (28)	0.97 ± 0.05 (29)	0.85 ± 0.06 (20)	0.92 ± 0.04 (49)	1.07 ± 0.04 (47)
Field sparrow	1.8 ± 0.23 (12)	1.8 ± 0.22 (5)	1.8 ± 0.13 (17)	1.9 ± 0.29 (15)	1.7 ± 0.16 (8)	1.8 ± 0.19 (23)	1.86 ± 0.09 (33)
Field sparrow[a]	–	–	–	1.9 ± 0.57 (4)	1.7 ± 0.16 (2)	1.8 ± 0.36 (6)	1.94 ± 0.07 (34)
Chipping sparrow	1.5 ± 0.23 (10)	1.1 ± 0.26 (4)	1.4 ± 0.14** (14)	2.4 ± 0.28 (18)	1.5 ± 0.16*** (11)	2.0 ± 0.20 (29)	2.3 ± 0.14 (15)
Chipping sparrow[a]	2.44 (1)	–	2.44 (1)	1.9 ± 0.57 (5)	1.48 (1)	1.8 ± 0.47 (6)	2.40 ± 0.09 (29)
Northern cardinal	1.2 ± 0.20 (8)	1.3 ± 0.33 (5)	1.2 ± 0.15 (13)	1.5 ± 0.11 (15)	1.5 ± 0.13 (14)	1.50 ± 0.08 (29)	1.45 ± 0.09 (39)
Northern cardinal[a]	–	0.4 ± 0.21 (2)	0.4 ± 0.21 (2)	1.14 ± 0.05 (8)	1.1 ± 0.10 (5)	1.14 ± 0.05 (13)	1.33 ± 0.08 (32)
Carolina chickadee	1.87 ± 0.09 (2)	2.5 ± 0.38 (8)	2.4 ± 0.32 (10)	–	2.7 ± 0.31 (8)	2.7 ± 0.31 (8)	2.7 ± 0.17 (37)
Downy woodpecker	6 ± 1.48 (4)	4.0 ± 0.86 (3)	4.9 ± 0.92 (7)	6 ± 1.09 (9)	6 ± 1.01 (5)	6.2 ± 0.77 (14)	5 ± 1.35 (12)
Downy woodpecker[a]	–	3.56 (1)	3.56 (1)	–	–	–	6.9 ± 0.83 (10)
Brown thrasher	0.4 ± 0.10 (3)	1.00 (1)	0.5 ± 0.17 (4)	0.5 ± 0.10 (5)	0.53 ± 0.08 (5)	0.50 ± 0.06 (10)	0.5 ± 0.10 (5)
Brown thrasher[a]	–	–	–	0.77 (1)	–	0.77 (1)	0.26 (1)
Song sparrow	3.0 ± 0.38 (2)	2.4 ± 0.10 (2)	2.7 ± 0.22 (4)	1.9 ± 0.11 (24)	2.2 ± 0.17 (4)	2.0 ± 0.10 (28)	2.0 ± 0.71 (2)
Song sparrow[a]	–	–	–	–	1.57 (1)	1.57 (1)	1.7 ± 0.28 (3)
Blue jay	0.87 ± 0.09 (2)	0.86 (1)	0.87 ± 0.05 (3)	0.9 ± 0.18 (2)	–	0.9 ± 0.18 (2)	1.0 ± 0.31 (2)
Indigo bunting	2.3 ± 0.16 (3)	–	2.3 ± 0.16 (3)	2.1 ± 0.31 (5)	0.90 (1)	1.9 ± 0.32 (6)	2.6 ± 0.19 (41)
Red-eyed vireo	1.03 (1)	0.7 ± 0.29 (2)	0.8 ± 0.20 (3)	–	1.0 ± 0.28 (3)	1.0 ± 0.28 (3)	0.97 ± 0.06 (59)
Wood thrush	3.50 (1)	1.7 ± 0.18 (2)	2.3 ± 0.62 (3)	–	1.89 ± 0.07 (3)	1.89 ± 0.07 (3)	2.4 ± 0.25 (19)
Common grackle	1.34 (1)	1.42 (1)	1.38 ± 0.04 (2)	1.0 ± 0.11 (10)	–	1.0 ± 0.11 (10)	1.30 ± 0.07 (5)
Common grackle[a]	–	–	–	1.2 ± 0.16 (6)	–	1.2 ± 0.16 (6)	1.39 ± 0.07 (20)
Blue-gray gnatcatcher	0.53 (1)	–	0.53 (1)	–	0.59 (1)	0.59 (1)	0.72 ± 0.09 (12)
House sparrow	–	–	–	0.76 ± 0.05 (12)	–	0.76 ± 0.05 (12)	–
House sparrow[a]	–	–	–	0.61 ± 0.04 (4)	–	0.61 ± 0.04 (4)	–
Northern mockingbird	–	–	–	1.1 ± 0.13 (7)	0.78 (1)	1.04 ± 0.12 (8)	1.4 ± 0.19 (8)
Northern mockingbird[a]	–	–	–	0.69 (1)	–	0.69 (1)	1.26 ± 0.07 (2)
Tufted titmouse	–	–	–	2.08 ± 0.09 (2)	1.47 ± 0.08 (6)	1.6 ± 0.12 (8)	1.80 ± 0.09 (36)
Carolina wren	–	–	–	1.1 ± 0.10 (5)	0.97 (1)	1.06 ± 0.09 (6)	0.71 ± 0.06 (25)
Brown-headed cowbird	–	–	–	1.9 ± 0.44 (3)	–	1.9 ± 0.44 (3)	1.92 ± 0.07 (51)

Table 3 continued

Common names	1999			2000			Reference sites (*n*)
	WVU (*n*)	USDA (*n*)	Both sites (*n*)	WVU (*n*)	USDA (*n*)	Both sites (*n*)	
Worm-eating warbler	–	–	–	2.2 ± 0.58 (2)	1.63 (1)	2.0 ± 0.39 (3	2.7 ± 0.24 (9)
House wren	–	–	–	1.3 ± 0.18 (2)	–	1.3 ± 0.18 (2)	1.1 ± 0.11 (9)
Scarlet tanager	–	–	–	–	4.03 (1)	4.03 (1)	2.8 ± 0.21 (17)
Yellow-breasted chat	–	–	–	1.89 (1)	–	1.89 (1)	2.1 ± 0.17 (4)

Mean ChE activities are expressed as micromoles AThChI (ChE substrate) hydrolyzed per minute per milliliter of plasma (μmol/min/mL)

Significantly different from the reference mean, * $P < 0.001$, ** $P < 0.0001$, natural log-transformed mean significantly different from reference mean, *** $P < 0.01$

[a] Juveniles

$\chi^2_1 = 0.13$, $P = 0.72$, $n = 814$ in 2000), so exposure frequency data were combined across sites within years. Exposure frequency was significantly higher among all birds at the treated orchard sites in each year compared to the reference sites, and was greater within the treated sites in 1999 than in 2000. Due to low numbers of some species in 2000, exposure frequency data were combined at the reference sites; and exposure was 4 % among all species at those sites. In 1999, 21 % of birds were exposed in the treated orchards ($\chi^2_1 = 88.41$, $P < 0.0001$, $n = 1{,}084$) and 14 % were exposed in 2000 ($\chi^2_1 = 51.89$, $P < 0.0001$, $n = 1{,}590$; Table 2). This equates to a rate of approximately one out of every five birds captured in 1999 and one out of every seven birds captured in 2000.

Species Differences

Species differences were tested first by comparing the frequency of exposure between the treated and reference sites in seven species for which a sufficient sample size for statistical analysis was obtained. These species were: American robin (*Turdus migratorius*), house finch (*Haemorhous mexicanus*), Northern cardinal (*Cardinalis cardinalis*), gray catbird (*Dumatella carolinensis*), American goldfinch (*Spinus tristis*), field sparrow (*Spizella pusilla*), and chipping sparrow (*Spizella passerina*; Table 2). Age groups (adults and juveniles) were combined within species, and species were combined across both treated sites. Justification for combining these data is given in Borges (2002). The proportions of exposed and unexposed individuals did not differ significantly between the seven species at the treated sites (Fisher's exact test, $P = 0.07$, $n = 265$ for 1999; $P = 0.28$, $n = 674$ for 2000) or at the reference sites (Fisher's exact test, $P = 0.99$, $n = 409$). In 1999, chipping sparrows and American robins demonstrated the highest exposure frequencies (47 and 29 %, respectively), whereas exposure was detected in fewer than 20 % of individuals in each of the other five species. When the proportion of exposed individuals was compared between treated and reference sites within each species, only chipping sparrows and American robins showed significantly higher proportions of exposed individuals at the treated sites compared to the reference sites (Fisher's exact test, $P = 0.0002$, $n = 145$ for robins; $P = 0.0001$, $n = 59$ for chipping sparrows, $\alpha = 0.007$). Chipping sparrows and American robins, respectively, also exhibited the highest and second highest proportions of exposed individuals in 2000 (Table 2; chipping sparrows: 20 %, American robins: 16 %). However, statistical comparisons of exposure frequency between treated and reference sites within these two species did not show any significant differences.

Species differences were also tested within years by comparing mean ChE activity between orchard and reference sites using ANOVA. Because of potential age differences in ChE activity (Gard and Hooper 1993), mean ChE activity was determined separately for adults and juveniles within a species (Table 3). Therefore, for discussion of this analysis and its results, these groups of birds are referred to as species-age groups. The above seven species used for analysis of the frequency of exposure were also used for statistical analysis of ChE activity. Because limited numbers of juveniles were captured for most species, only adults of six of these seven species were included in the analysis. Therefore, statistical analyses based on mean ChE activity involved eight species-age groups: adult house finch, Northern cardinal, gray catbird, American goldfinch, field sparrow, and chipping sparrow; and adult and juvenile American robins. Although some analyses required a transformation to meet the assumptions of the statistical tests, untransformed means of ChE activity are presented in Table 3.

In 1999, 15 out of the 21 species–age groups had lower mean ChE activity in at least one treated-orchard site compared to the reference sites, while six showed equal or higher ChE activity (Table 3). For the 1999 statistical analyses involving the eight species–age groups, ANOVA indicated no site by species–age group interaction ($F_{7,109} = 0.62$, $P = 0.74$), and no site effects related to the two treated orchard sites, so the data for each species–age group were combined across the treated-orchard sites. Mean ChE was lower at the treated-orchard sites compared to the reference sites for all species–age groups except house finches, whose mean ChE activity was 8.7 % higher within the treated orchards. The degree of ChE depression was significant only among adult chipping sparrows ($F_{1,125} = 21.98$, $P < 0.0001$, $\alpha = 0.00625$) and adult American goldfinches ($F_{1,394} = 9.42$, $P = 0.0023$, $\alpha = 0.00625$), whose orchard site means were 39.1 and 18.7 % below the reference means, respectively. ChE activity depression in other species–age groups included in the statistical analysis ranged from 3.2 to 16.5 % below their respective reference means.

Mean ChE activity was lower at one or both treated orchard sites compared to the reference sites for 22 of 34 species–age groups in 2000, while 12 showed equal or higher ChE activity (Table 3). The data from 2000 were natural log transformed for statistical analysis, but untransformed means are presented in Table 3. The same species–age groups as used above were used for analysis of the mean ChE activity data from 2000. A significant difference in mean ChE activity was present between the USDA- and WVU-treated orchard sites ($F_{1,618} = 4.01$, $P = 0.0457$); this was driven by a large difference in mean ChE activity for chipping sparrows between the two orchard sites. Therefore, an ANOVA was performed for adult chipping sparrows only, comparing log mean ChE activity separately between the USDA- and WVU-treated sites to the reference, and a separate ANOVA was performed with the remaining species–age groups comparing log mean ChE activity calculated from both treated orchard sites combined to the reference sites (similar to the 1999 analyses). ChE activity for adult chipping sparrows was significantly lower only at USDA ($|t| = 3.72$, $P = 0.0017$, $n = 41$), where log mean ChE activity (0.33 ± 0.1) was 39.0 % below the log mean for the reference sites (0.82 ± 0.09). Log mean ChE activity was only 3.4 % lower at WVU (0.79 ± 0.08) compared to the reference. ChE activity was lower at the treated orchard sites compared to the reference sites for nearly all of the remaining species–age groups, ranging 1.2–14.8 % below each species–age group's respective reference mean. The exception occurred in adult Northern cardinals, whose log mean ChE activity was 2.8 % higher at the treated-orchard sites compared to the reference sites. However, none of these relationships were significant.

Discussion

Many studies of pesticide effects on birds have been conducted in conventionally-managed fruit orchards (e.g., Bishop et al. 2000; Bouvier et al. 2011; Graham and DesGranges 1993; Johnson et al. 1976). These environments present complex exposure scenarios for birds because orchards receive multiple pesticides per application and multiple applications per season. Additionally, orchards attract many species of birds because they provide habitat for nesting and other resources (Bishop et al. 2000; Boutin et al. 1999; Bouvier et al. 2011; Burgess et al. 1999; Fluetsch and Sparling 1994; Johnson et al. 1976; Wilson et al. 2001). Anti-ChE insecticides, which inhibit acetylcholinesterase, are still used in these and other agricultural environments, and because of their toxicity and non-selective mode of action, adverse effects have been observed over a wide taxonomic spectrum, including birds (e.g., Elliott et al. 2008; Gill et al. 2004; Parsons et al. 2010; Strum et al. 2010). Because the occurrence of anti-ChE insecticide exposure in birds can be determined non-destructively (Fildes et al. 2009; Gard and Hooper 1993; Hill and Fleming 1982; Hooper et al. 1989; Hunt and Hooper 1993; Vergara et al. 2008), study of these compounds in orchard-dwelling birds provided an avenue by which avian pesticide exposure was explored across many species in the current study.

We detected significant exposure among free-ranging birds at our treated orchard sites. We were not able to detect

any specific pesticide-related trends in exposure using our data, since too many chemicals were present at any time to make this comparison possible. Table 1 provides a listing of anti-ChEs present during sampling, but it does not list the herbicides, fungicides, fertilizers, miticides, acaracides, and adjuvants that were present along with these insecticides. We noted an apparent year difference in our results, which may be attributable to the cancellation of methyl parathion applications in fruit orchards after 1999 (USEPA 1999). The toxicity of this chemical to birds is well documented (Fleischli et al. 2004; Robinson et al. 1988; Smith 1987; USEPA 2006). However, in 1999 the region also experienced a drought with high temperatures, whereas in 2000 the weather was cooler and wetter. Birds exposed to anti-ChEs in 1999 may have been more physiologically stressed by high temperatures, lack of water, and/or drought-related decreased food supply, which may have affected their sensitivity (Grue et al. 1983; Rattner and Fairbrother 1991). The wetter conditions in 2000 may have also diluted the pesticides, increased their chemical breakdown, or washed them away from our field sites. Neither of the treated sites had widespread irrigation, so it is not likely that more birds were attracted to them for this reason.

The exposure that we detected clearly differed among species, and our data show that some species of birds that we studied were better indicators of exposure to anti-ChEs than others at our study sites. Had we selected a priori only one or two passerine species as indicator species based on their abundance and ease of capture, the most likely candidates would have been our two most frequently captured species, American robins and gray catbirds. However, in terms of exposure frequency, American robins did not show greater exposure than any other species included in statistical analyses and gray catbirds varied in their exposure, even showing no significant exposure in 1 year. Furthermore, neither species had significantly reduced ChE activity in the treated orchards compared to their conspecifics in the reference sites in either year. Chipping sparrows were the least numerous among the seven species we included in statistical analyses and, therefore, would have been unlikely to be considered as indicator species at these sites. However, they consistently showed higher exposure frequencies than other species (except for one site in 2000) and greater mean ChE activity reduction. Thus, chipping sparrows appear to be better indicators of exposure than the presumptive species (American robins and gray catbirds). We conducted a study on anti-ChE exposure to birds in orchards using the presumptive indicator species, our results would have shown that passerines and similar birds were not significantly exposed in these orchards based on our methods of analysis.

To our knowledge, a method of choosing indicators for study of pesticides has not been clearly laid out. Greig-Smith (1990) outlines general guidance and offers six possible categories of indicator species choice based on the likelihood of exposure, expected sensitivity, degree of impact to a particular species, representativeness of typical characteristics of other species in the community, abundance, and ease of effects measurements. This guidance also cautions that not all of these criteria could be easily predicted, and that it may be desirable to study species from several categories. Except in cases where the impact to a particular species is of interest (e.g., a species that is endangered or otherwise of significant concern), our results show that none of these is necessarily reliable as a sole approach to choosing an indicator species. Therefore, some additional guidance on the choice of indicator species for studies with these compounds is needed.

One approach may be to utilize previously collected data, such as pesticide incidents and published field or laboratory pesticide studies. However, incident information can be biased toward conspicuous species (Vyas 1999), and many factors may affect the sensitivity of a species in the field compared to the laboratory (Vyas et al. 2006). Knowledge gleaned from published studies like this one can also be useful, but care must be taken to ensure that a species that has been studied previously is appropriate given the pesticide of interest, exposure routes, season, study site environment, and geographic location. It is important also to consider all data available on a species and the influence of the methods used. For example, we relied solely on plasma ChE activity or plasma ChE reactivation, which do not indicate the degree of exposure or potential severity of effects, and our capture methods caught only birds that were able to fly (and presumably less affected than other birds). Reliance on only this method introduces a collection bias, which is a common problem in field studies that utilize free-ranging wildlife to examine pesticide effects (Fryday et al. 1996; Mineau and Peakall 1987). Birds may be less able to fly or may seek cover when sickened (Mineau and Peakall 1987; Vyas 1999), thereby preventing capture by our methods. This bias presents some difficulty in interpreting our findings. For example, overall exposure in the orchard may be higher than detected in our study because we were not able to capture the more greatly affected individuals. Additionally, American robins may have been heavily exposed and adversely affected, but due to their abundance they were quickly replaced by less-affected floater individuals from surrounding areas (Hensley and Cope 1951; Stewart and Aldrich 1951). It is also possible that chipping sparrows are less sensitive to the anti-ChE exposures, and therefore their lower abundance may not be a reflection of adverse effects but an artifact of other aspects of their ecology (e.g., habitat preferences). Robins have frequently been the focus of orchard pesticide studies, and significant effects have been

observed in this species (Cobb et al. 2000; Fluetsch and Sparling 1994; Gill et al. 2000; Graham and DesGranges 1993). However, Graham and DesGranges (1993) and Rondeau and DesGranges (1995) both reported significant plasma ChE depression in both chipping sparrows and robins in areas treated with OPs, but chipping sparrows were the only species to demonstrate significant brain ChE depression in Christmas tree plantations. The findings of these studies corroborate some of our results and contrast others. Therefore, reliance on previously collected data to focus species choice requires consideration of many factors that may have influenced the results.

Since the selection of indicator species for research is critical, and little species-specific information is currently available to aid in selection, greater focus on multiple-species approaches would benefit researchers studying pesticide effects in the field. Full-scale studies with many species would yield valuable information on field effects, especially at the community level, but they are resource intensive. However, a study similar to ours conducted as a preliminary activity can characterize exposure at the avian community level, as well as refine directions for future research by identifying possible at risk species and by directing the experimental design. Additional observations, such as brain ChE analyses, behavioral observations, or supplemental pesticide residue analyses (e.g., on skin and feathers, see Vyas et al. 2007) would have aided in the interpretation of the implications of our data on species-specific exposure. Therefore, such a pilot study would benefit from the use of multiple measurements of exposure and effects, as well as multiple capture or observation methods, so as to avoid potential sampling bias and characterize more fully the potential effects of exposure. This approach would be an invaluable tool for selecting indicator species based on biological relevance, even if the amount of data collected in the pilot study could not be used in rigorous statistical analyses. Our study demonstrates the value of conducting multi-species screening to support the selection of indicator species for research.

Acknowledgments The authors thank K. Bowser, K. Crawford, J. Davignon, W. Echard, G. Gentry, S. Gentry, M. Hamilton, S. Han, J. Healy, T. Johnson, M. Kohns, N. Martin, R. Nichols, W. Potter, S. Rush, J. Spann, S. Wagner, K. Waite, and M. Wilcox for assistance in the field, laboratory, or with other aspects of this project. We also thank M. Brown and H. Hogmire for providing guidance and access to the WVU and USDA orchards; D. Leach, D. Magaha, J. Hendricks, and the farm crews at the WVU and USDA orchards for aid in coordinating field work at the WVU and USDA sites, L. Kidwell for assistance at the Paw Paw site, and to C. Hill and P. Hott for allowing access to their land. Thanks also to M. Hooper for technical assistance with ChE analyses as well as data used in this project, and to N. Beyer, G. Heinz, and two anonymous reviewers for comments to improve the manuscript. This project was funded by Patuxent Wildlife Research Center with funds received through an Interagency Agreement (DW14937610-01-0) between the U.S. Environmental Protection Agency (EPA) and the U.S. Geological Survey. Funding was also provided by the Sigma Xi Scientific Research Society, through their Committee on Grants-in-Aid of Research. The experiments associated with this research comply with the current laws of the US.

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References

Andelman SJ, Fagan WF (2000) Umbrellas and flagships: efficient conservation surrogates or expensive mistakes. Proc Natl Acad Sci USA 97:5954–5959

Bani L, Massimino D, Bottoni L, Massa R (2006) A multiscale method for selecting indicator species and priority conservation areas: a case study for broadleaved forests in Lombardy, Italy. Conserv Biol 20:512–526

Bishop CA, Collins B, Mineau P, Burgess NM, Read WF, Risley C (2000) Reproduction of cavity-nesting birds in pesticide-sprayed apple orchards in southern Ontario, Canada, 1988–1994. Environ Toxicol Chem 19:588–599

Borges SL (2002) Exposure of free-flying birds to anticholinesterase insecticides in two conventionally managed fruit orchards. MS Thesis, University of Maryland

Boutin C, Freemark KE, Kirk DA (1999) Farmland birds in southern Ontario: field use, activity patterns and vulnerability to pesticide use. Agric Ecosyst Environ 72:239–254

Bouvier J-C, Toubon J-F, Boivin T, Sauphanor B (2005) Effects of apple orchard management strategies on the great tit (*Parus major*) in southeastern France. Environ Toxicol Chem 24:2846–2852

Bouvier J-C, Ricci B, Agerberg J, Lavigne C (2011) Apple orchard pest control strategies affect bird communities in southeastern France. Environ Toxicol Chem 30:212–219

Burgess NM, Hunt KA, Bishop C, Weseloh DV (1999) Cholinesterase inhibition in tree swallows (*Tachycineta bicolor*) and Eastern bluebirds (*Sialia sialis*) exposed to organophosphorus insecticides in apple orchards in Ontario, Canada. Environ Toxicol Chem 18:708–716

Cobb GP, Mellott R, Brewer LW, Bens CM, Kendall RJ (2000) Diazinon dissipation from vegetation, occurrence in earthworms, and presence in avian gastrointestinal tracts collected from apple orchards following D-Z-N® 50W application. Environ Toxicol Chem 19:1360–1367

Elliott JE, Birmingham AL, Wilson LK, McAdie M, Trudeau S, Mineau P (2008) Fonofos poisons raptors and waterfowl several months after granular application. Environ Toxicol Chem 27:452–460

Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Fildes KJ, Szabo JK, Astheimer L, Hooper M, Buttemer WA (2009) Plasma cholinesterase characteristics in native Australian birds: significance for monitoring avian species for pesticide exposure. Emu 109:41–47

Fleischli MA, Franson JC, Thomas NJ, Finley DL, Riley W Jr (2004) Avian mortality events in the United States caused by anticholinesterase pesticides: a retrospective summary of National

Wildlife Health Center records from 1980 to 2000. Arch Environ Contam Toxicol 46:542–550
Fluetsch KM, Sparling DW (1994) Avian nesting success and diversity in conventionally and organically managed apple orchards. Environ Toxicol Chem 13:1651–1659
Fryday SL, Hart ADM, Langton SD (1996) Effects of exposure to an organophosphorus pesticide on the behavior and use of cover by captive starlings. Environ Toxicol Chem 15:1590–1596
Gard NW, Hooper MJ (1993) Age-dependent changes in plasma and brain cholinesterase activities of Eastern bluebirds and European starlings. J Wildl Dis 29:1–7
Gill H, Wilson LK, Cheng KM, Trudeau S, Elliott JE (2000) Effects of azinphos-methyl on American robins breeding in fruit orchards. Bull Environ Contam Toxicol 65:756–763
Gill H, Williams TD, Bishop CA, Cheng KM, Elliot JE (2004) Effects of azinphos-methyl on cholinergic responses and general health in zebra finches (*Taeniopygia guttata*) after previous treatment with *p,p′*-DDE. Arch Environ Contam Toxicol 48:118–126
Graham DJ, DesGranges J (1993) Effects of the organophosphate azinphos-methyl on birds of potato fields and apple orchards in Quebec, Canada. Agric Ecosyst Environ 43:183–199
Greig-Smith PW (1990) Intensive study versus extensive monitoring in pesticide field trails. In: Somerville L, Walker CH (eds) Pesticide effects of terrestrial wildlife. Taylor and Francis, London, pp 217–239
Grue CE, Fleming WJ, Busby DG, Hill EF (1983) Assessing hazards of organophosphate pesticides to wildlife. Trans N Am Wildl Nat Resour Conf 48:200–220
Hardy AR (1990) Estimating exposure: the identification of species at risk and routes of exposure. In: Somerville L, Walker CH (eds) Pesticide effects of terrestrial wildlife. Taylor and Francis, London, pp 81–97
Hensley MM, Cope JB (1951) Further data on removal and repopulation of the breeding birds in a spruce-fir forest community. Auk 68:483–493
Hill EF (1992) Avian toxicology of anticholinesterases. In: Ballantyne B, Marrs TC (eds) Clinical and experimental toxicology of organophosphates and carbamates. Butterworth-Heinemann Ltd., Oxford, pp 272–293
Hill EF, Fleming WJ (1982) Anticholinesterase poisoning of birds: field monitoring and diagnosis of acute poisoning. Environ Toxicol Chem 1:27–38
Hooper MJ, Detrich PJ, Weisskopf CP, Wilson BW (1989) Organophosphorus insecticide exposure in hawks inhabiting orchards during winter dormant-spraying. Bull Environ Contam Toxicol 42:651–659
Hunt KA, Hooper MJ (1993) Development and optimization of reactivation techniques for carbamate-inhibited brain and plasma cholinesterases in birds and mammals. Anal Biochem 212:335–343
Hutto RL (1998) Using landbirds as an indicator species group. In: Marzluff JM, Sallabanks R (eds) Avian conservation: research and management. Island Press, Washington, DC, pp 75–92
Johnson EV, Mack GL, Thompson DQ (1976) The effects of orchard pesticide applications on breeding robins. Wilson Bull 88:16–35
Jones J (2003) Tree swallows (*Tachycineta bicolor*): a new model organism? Auk 120:591–599
Landres PB, Verner J, Thomas JW (1988) Ecological uses of vertebrate indicator species: a critique. Conserv Biol 2:316–328
Millikin RL, Smith JNM (1990) Sublethal effects of fenitrothion on forest passerines. J Appl Ecol 27:983–1000
Mineau P, Peakall DB (1987) An evaluation of avian impact assessment techniques following broad-scale forest insecticide sprays. Environ Toxicol Chem 6:781–791
Mineau P, Fletcher MR, Glaser LC, Thomas NJ, Brassard C, Wilson LK, Elliott JE, Lyon LA, Henny CJ, Bollinger T, Porter SL (1999) Poisoning of raptors with organophosphorus and carbamate pesticides with emphasis on Canada, U.S., and U.K. J Raptor Res 33:1–37
Moore NW (1966) A pesticide monitoring system with special reference to the selection of indicator species. J Appl Ecol 1966:261–269
Niemi GJ, McDonald ME (2004) Application of ecological indicators. Annu Rev Ecol Evol Syst 35:89–111
Parsons KC, Mineau P, Renfrew RB (2010) Effects of pesticide use in rice fields on birds. Waterbirds 33(sp1):193–218
Patnode KA, White DH (1991) Effects of pesticides on songbird productivity in conjunction with pecan cultivation in southern Georgia: a multiple-exposure experimental design. Environ Toxicol Chem 10:1479–1486
Rattner BA, Fairbrother A (1991) Biological variability and the influence of stress on cholinesterase activity. In: Mineau P (ed) Cholinesterase-inhibiting insecticides: their impact on wildlife and the environment. Elsevier Science Publishers, New York, pp 35–71
Robinson SC, Kendall RJ, Robinson R, Driver CJ, Lacher TD Jr (1988) Effects of agricultural spraying of methyl parathion on cholinesterase activity and reproductive success in wild starlings (*Sturnus vulgaris*). Environ Toxicol Chem 7:343–349
Rondeau G, DesGranges J (1995) Effects of insecticide use on breeding birds in Christmas tree plantations in Quebec. Ecotoxicology 4:281–298
Smith GJ (1987) Pesticide use and toxicology in relation to wildlife: organophosphorus and carbamate compounds. U.S. Fish and Wildlife Service Resource Publication 170
Stewart RE, Aldrich JW (1951) Removal and repopulation of breeding birds in a spruce-fir forest community. Auk 68:471–482
Stinson ER, Hayes LE, Bush PB, White DH (1994) Carbofuran affects wildlife on Virginia corn fields. Wildl Soc Bull 22:566–575
Stone WB (1979) Poisoning of wild birds by organophosphate and carbamate pesticides. NY Fish Game J 26:38–47
Strum KM, Hooper MJ, Johnson KA, Lanctot RB, Zacagnini ME, Sandercock BK (2010) Exposure of nonbreeding migratory shorebirds to cholinesterase inhibiting contaminants in the Western hemisphere. Condor 112:15–28
Thompson ID (2006) Monitoring of biodiversity indicators in boreal forests: a need for improved focus. Environ Monit Assess 121:263–273
Thompson HM, Langton SD, Hart ADM (1995) Prediction of interspecies differences in the toxicity of organophosphorus pesticides to wildlife—a biochemical approach. Comp Biochem Physiol 111C:1–12
U.S. Environmental Protection Agency (USEPA) (1992) Personal protective equipment. US Code of Federal Regulations, Title 40, Part 170, Section 240, as amended Sep 1, 2004. US Government Printing Office, Washington, DC
USEPA (1999) Methyl parathion, receipt of requests for cancellation; cancellation order. Federal Register vol. 64, No 207, Oct 27. USEPA, Washington, DC, pp 5787–5788
USEPA (2006) Reregistration eligibility decision for methyl parathion. http://www.epa.gov/pesticides/reregistration/REDs/methyl_parathion_red.pdf. Accessed 15 Oct 2013
Vergara P, Fargallo JA, Banda E, Parejo D, Lemus JA, Garcia-Montijano M (2008) Low frequency of anti-acetylcholinesterase pesticide poisoning in lesser and Eurasian kestrels in Spanish grassland and farmland populations. Biol Conserv 141:499–505
Vyas NB (1999) Factors influencing the estimation of pesticide-related wildlife mortality. J Toxicol Ind Health 15:186–191
Vyas NB, Spann JW, Hulse CS, Borges SL, Bennett RS, Torrez M, Williams BI, Leffel R (2006) Field evaluation of an avian risk assessment model. Environ Toxicol Chem 25:1762–1771

Vyas NB, Spann JW, Hulse CS, Gentry S, Borges SL (2007) Dermal insecticide residues from birds inhabiting an orchard. Environ Monit Assess 133:209–214

Wiemeyer SN, Sparling DW (1991) Acute toxicity of four anticholinesterase insecticides to American kestrels, Eastern screech-owls and Northern bobwhites. Environ Toxicol Chem 10:1139–1148

Wilson L, Martin PA, Elliott JE, Mineau P, Cheng KM (2001) Exposure of California quail to organophosphorus insecticides in apple orchards in the Okanagan Valley, British Columbia. Ecotoxicology 10:79–90